Developmental Biology 322 (2008) 322–331

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

SKR-1, a homolog of Skp1 and a member of the SCF^{SEL-10} complex, regulates sex-determination and LIN-12/Notch signaling in *C. elegans*

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ARTICLE INFO

Article history:
Received for publication 5 June 2008
Revised 29 July 2008
Accepted 30 July 2008
Available online 7 August 2008

Keywords:
SCF
Sex-determination
C. elegans
skr-1
sel-10
cul-1

ABSTRACT

Sex-determination in *Caenorhabditis elegans* requires regulation of gene transcription and protein activity and stability. *sel-10* encodes a WD40-repeat-containing F-box protein that likely mediates the ubiquitin-mediated degradation of important sex-determination factors. Loss of *sel-10* results in a mild masculinization of hermaphrodites, whereas dominant alleles of *sel-10,* such as *sel-10(n1074)*, cause a more severe masculinization, including a reversal of the life versus death decision in sex-specific neurons. To investigate about how *sel-10* regulates sex-determination, we conducted a *sel-10(n1074)* suppressor screen and isolated a weak loss-of-function allele of *skr-1*, one of 21 Skp1-related genes in *C. elegans*. Skp1, Cullin, and F-box proteins, such as SEL-10, are components of the SCF E3 ubiquitin-ligase complex. We present genetic evidence that the *sel-10(n1074)* masculinization phenotype is dependent upon *skr-1* and *cul-1* activity. Furthermore, we show that the SKR-1(M140I) weak loss-of-function mutation interferes with SKR-1/SEL-10 binding. Unexpectedly, we found that the G567E substitution in SEL-10 caused by the *n1074* allele impairs the binding of SEL-10 to SKR-1 and the dimerization of SEL-10, which may be important for SEL-10 function. Our results suggest that SKR-1, CUL-1 and SEL-10 constitute an SCF E3 ligase complex that plays an important role in modulating sex-determination and LIN-12/Notch signaling in *C. elegans*.

Introduction

Somatic sexual differentiation in *Caenorhabditis elegans* is controlled by a negative regulatory cascade that involves signal transduction, transcriptional and translational regulation, and targeted protein degradation (Fig. 1; Zarkower, 2006). The initial determinant of sex in *C. elegans* is the X chromosome to autosome ratio whereby XX animals (1:1 X:A ratio) develop as hermaphrodites and XO animals (1:2 X:A ratio) develop as males (Hodgkin, 1987; Madl and Herman, 1979). This X to autosome counting mechanism (see Meyer, 2000 for review) regulates the male-specific expression of *her-1* (Hermaphrodization), which encodes a secreted protein that promotes male development (DeLong et al., 1993; Perry et al., 1993; Trent et al., 1991). HER-1 inhibits the activity of a transmembrane receptor TRA-2 (Transformer) in males, while TRA-2 remains active in hermaphrodites (Kuwabara et al., 1992). TRA-2 promotes hermaphrodite development by inhibiting the activity of the FEM (Feminization) protein complex composed of an ankyrin-repeat protein FEM-1 (Spence et al., 1990), a type 2C protein phosphatase FEM-2 (Chin-Sang and Spence, 1996; Pilgrim et al., 1995), a novel protein FEM-3 (Ahringer et al., 1992), and a Cullin, CUL-2 (Starostina et al., 2007). The FEM proteins and CUL-2 form a CBC (Cul2, Elongin B, Elongin C) E3 ubiquitin-ligase complex referred to as CBC^{FEM-1}, which promotes male development by targeting TRA-1 for degradation (Starostina et al., 2007). TRA-1, a DNA-binding Zinc finger protein, promotes hermaphrodite development by repressing the expression of male-specific genes in hermaphrodites (Conradt and Horvitz, 1998; Yi et al., 2000; Zarkower and Hodgkin, 1992). TRA-1 is often referred to as a master regulator of sex-determination, since loss of *tra-1* results in the transformation of XX animals into fertile males (Hodgkin, 1987). Similarly, loss of any of the other core sex-determination pathway components results in a complete or near complete sexual transformation. However, there are components of the sex-determination pathway that do not act as switches between the male and hermaphrodite fate but rather "fine-tune" the pathway by targeting core pathway proteins for ubiquitin-mediated degradation.

SEL-10 is an F-box protein with eight WD40-repeats and may serve as a substrate recognition component of the SCF (Skp1-Cullin-F-box) ubiquitin-ligase complex that targets substrate proteins for ubiquitin-mediated proteolysis by the proteasome (for a review on SCF complexes see Cardozo and Pagano, 2004). SEL-10 was first identified for its role in the Notch pathway and vulval development in *C. elegans* by targeting LIN-12/Notch and SEL-12/Presenilin for

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doi:10.1016/j.ydbio.2008.07.035

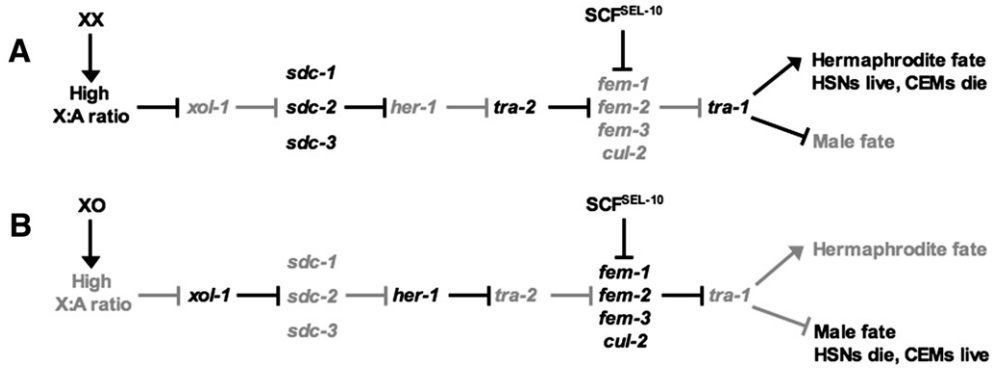

Fig. 1. A simplified view of the sex-determination pathway in XX animals (A) and XO animals (B). Sex-determination genes depicted in black are active and genes depicted in grey are repressed (see text for detail). SCF^{SEL-10} is an E3 ubiquitin ligase composed of SKR-1, CUL-1, and SEL-10.

degradation (Hubbard et al., 1997; Wu et al., 1998). SEL-10 was also reported to affect sex-determination by mediating the turnover of the male-promoting proteins FEM-1 and FEM-3 (Fig. 1). *sel-10(null)* hermaphrodites are not visibly masculinized but exhibit a synergistic masculinization phenotype in combination with weak alleles of *tra-2* (Jager et al., 2004). However, dominant mutations in *sel-10*, such as *sel-10(n1074)*, cause a stronger masculinization phenotype in hermaphrodites, characterized by the reversal of the cell death fates of the sex-specific neurons: the HSNs and CEMs (Desai and Horvitz, 1989; Jager et al., 2004). The HSNs (hermaphrodite-specific neurons) control vulval muscle contraction and egg laying in hermaphrodites and are eliminated through programmed cell death (PCD) in male embryos. Mutations that cause inappropriate HSN death in hermaphrodites render the animals egg laying defective (Egl) and they become bloated with eggs (Desai et al., 1988; Desai and Horvitz, 1989; Sulston, 1976). The CEMs (cephalic male neurons), which are involved in mediating chemotaxis of males toward hermaphrodites for mating (White et al., 2007), are born in both sexes but are selectively removed through programmed cell death (PCD) in hermaphrodite embryos (Sulston and Horvitz, 1977).

n1074 and seven other dominant alleles of *sel-10* cause the same single nucleotide change that results in Glycine to Glutamate substitution at amino acid 567 (G567E) in the carboxyl terminus of SEL-10, located in the eighth WD40-repeat region (Jager et al., 2004; Orlicky et al., 2003). It is unclear how SEL-10(G567E) affects the activity of the SCF^{SEL-10} complex to cause a dominant, masculinization defect. We thus carried out a *sel-10(n1074)* suppressor screen to identify components that may act with SEL-10 to regulate sex-determination.

Here we report the identification of *skr-1* (Skp1-related *1*) as a co-factor of *sel-10*. We isolated a weak loss-of-function mutation in *skr-1*, *sm151* that specifically suppresses the masculinization defect of *sel-10 (n1074)* animals and causes a Methionine to Isoleucine substitution at amino acid 140 (M140I). SKR-1 is a *C. elegans* homolog of the human Skp1 protein, a member of the SCF complex that targets substrate proteins for ubiquitin-mediated degradation by the proteasome (Bai et al., 1996; Cardozo and Pagano, 2004; Feldman et al., 1997; Skowyra et al., 1997; Zhang et al., 1995). We found that SEL-10 binds SKR-1 and this binding is compromised by the SKR-1(M140I) mutation. The SKR-1/SEL-10 binding is further reduced in the presence of both SKR-1 (M140I) and SEL-10(G567E) mutations. These results suggest that SEL-10 and SKR-1 likely act in the same SCF complex and provide a mechanistic basis for the suppression of the *sel-10(n1074)* masculinization phenotype by *skr-1(sm151)*. Our study also reveals an important and unexpected role for the C-terminal tail of SEL-10 in SKR-1 binding, SEL-10 dimerization, and in regulating the activity of the SCF E3 ligase complex.

Materials and methods

Strains and genetic manipulations

Strains were derived from the Bristol strain N2, grown at 20°C unless otherwise noted, and constructed using standard procedures (Brenner, 1974). Mutations used are from Brenner (1974) unless noted and are listed by linkage group (LG). LGI: *dpy-5(e61)*, *unc-29(e193)*, *unc-75(e950)*, *skr-1(sm151) (tm2391)* (this work). LGII: *smIs23[pkd-2::GFP]* (Peden et al., 2007), *lin-23(e1883)*, *lin-23(ot1)* (Mehta et al., 2004), *tra-2(n1106)* (Desai and Horvitz, 1989). LGIII: *lin-12(n302)* (Greenwald et al., 1983), *lin-12(ar170)* (Hubbard et al., 1997), *cul-1 (e1756)*, *tra-1(e1488)*. LGIV: *smIs26[pkd-2::GFP tph-1::GFP unc-76(+)]* (Peden et al., 2007), *dpy-20(e1282)*. LGV: *egl-1(n1084)* (Conradt and Horvitz, 1999), *sel-10(ar41)* (Hubbard et al., 1997), *(n1074)* (Desai and Horvitz, 1989), *(ok1632)* (gift of the *C. elegans* knockout consortium, Oklahoma Medical Research Foundation), *him-5(e1490)*, *unc-76 (e911)*. *hT2[qIs48]* (hereafter *hT2[GFP]*) (Miskowski et al., 2001) was used as a balancer for LGI and III. *qIs48* is an insertion of *ccEx9747* with markers: *myo-2::GFP* expressed in the pharynx, *pes-10::GFP* expressed in embryos, and a gut promoter driving GFP in the intestine.

We sequenced the *sel-10(ok1632)* deletion and found a 898 base pair deletion between the 55th and 953rd base pairs after the start. There is also a 15 base pair insertion (gtattatctagtatt). The conceptual translation of *sel-10(ok1632)* is predicted to cause a truncation of the protein after the first 18 amino acids and thus is likely to represent a null allele.

Isolation, mapping, and cloning of skr-1(sm151)

smIs23; *dpy-20(e1282)*; *sel-10(n1074)* hermaphrodites were mutagenized with 50 mM EMS and F_2 non-Egl animals lacking CEMs were isolated as suppressors. From 21,800 mutagenized genomes, 5 extragenic suppressors including *sm151* were isolated. *sm151* was mapped to an interval on LGI based on standard three-factor mapping. *sel-10(n1074)* was homozygous in all mapping strains. *sm151* was mapped to the right of *unc-29* (genetic position 3.29) based on mapping with *dpy-5(e61) unc-29(e193)*. 11/11 Dpy non-Unc and 0/6 Unc non-Dpy animals segregated *sm151*. *sm151* was then mapped to position 3.63 between *dpy-5* (0.0) and *unc-75* (9.44). 9/28 Dpy non-Unc and 5/11 Unc non-Dpy animals segregated *sm151*.

Since *sm151* mapped near the *skr-2* and *skr-1* loci (3.77), they were tested as candidates. A 4704 base pair fragment of the *skr-2 skr-1* region was amplified by PCR including 1430 base pairs upstream of the *skr-2* start codon to 654 base pairs downstream of the *skr-1* stop codon. *sm151*; *smIs23*; *sel-10(n1074)* hermaphrodites were injected with the *skr-2 skr-1* PCR fragment at 20 ng/μl and *rol-6(su1006)* at 60 ng/μl. 3/3 transgenic lines exhibited rescue. To determine whether

skr-2 or *skr*-1 rescued, two separate PCR fragments were generated and tested for rescue. A 2405 base pair fragment of *skr-1* was amplified by PCR including 991 base pairs upstream of the start and 654 base pairs downstream of the stop codon. Similarly, a 3290 base pair fragment of *skr-2* was amplified by PCR including 1430 base pairs upstream of the start and 1109 base pairs downstream of the stop codon. 3/3 transgenic lines carrying *skr-1* rescued whereas 0/3 transgenic lines carrying *skr-2* rescued (Fig. 2).

The *skr-1* locus was amplified by PCR from *skr-1(sm151)*; *smIs23*; *sel-10(n1074)* animals and the entire open reading frame was sequenced. A single base pair change was identified 649 base pairs downstream of the start resulting in a codon change of ATG to ATA and an amino acid change at Methionine 140 to Isoleucine (Fig. 2).

Isolation of skr-1(tm2391)

The *skr-1* deletion allele (*tm2391)* was isolated from pools of worms mutagenized by UV/trimethylpsoralen. A primer pair (Forward: CGCATCATACGACACACTCA; Reverse: AGGACAATGTGTGAAGTGTG) and a nested primer pair (Forward: ATCCGAGGCGGCAAAGGAAC; Reverse: GGGTAATTTAATCCTCGCAC) were used for PCR screening of the deletion allele. *skr-1(tm2391)* is a 435 base pair deletion that deletes from the 140th base pair through the 575th base pair after the *skr-1* ATG start codon. This deletes the entire second exon and part of the first and second introns of *skr-1* and is a predicted null allele (Fig. 2). To prove that this deletion allele is specific to *skr-1* and does not affect *skr-2*, we rescued the *skr-1(tm2391)* mutant with an extra-chromosomal array carrying *skr-1(+)*. 2/2 lines rescued somatic phenotypes associated with *skr-1(tm2391)* such as larval lethality and hyperplasia of somatic gonad tissues. Sterility was not rescued as transgenes are often silenced in the germline in *C. elegans* (Kelly et al., 1997).

Yeast two-hybrid assays

The following cDNAs were used for yeast two-hybrid vector construction: *skr-1* (yk1092h10), *sel-10* (yk21f12) (gifts of Y. Kohara). cDNAs were PCR amplified with Gateway *attB*1 and *attB2* primers and inserted into pDONR221 (Invitrogen, Carlsbad, CA) to create cDNA entry clones. Mutant cDNAs corresponding to *skr-1(sm151)* and *sel-10*

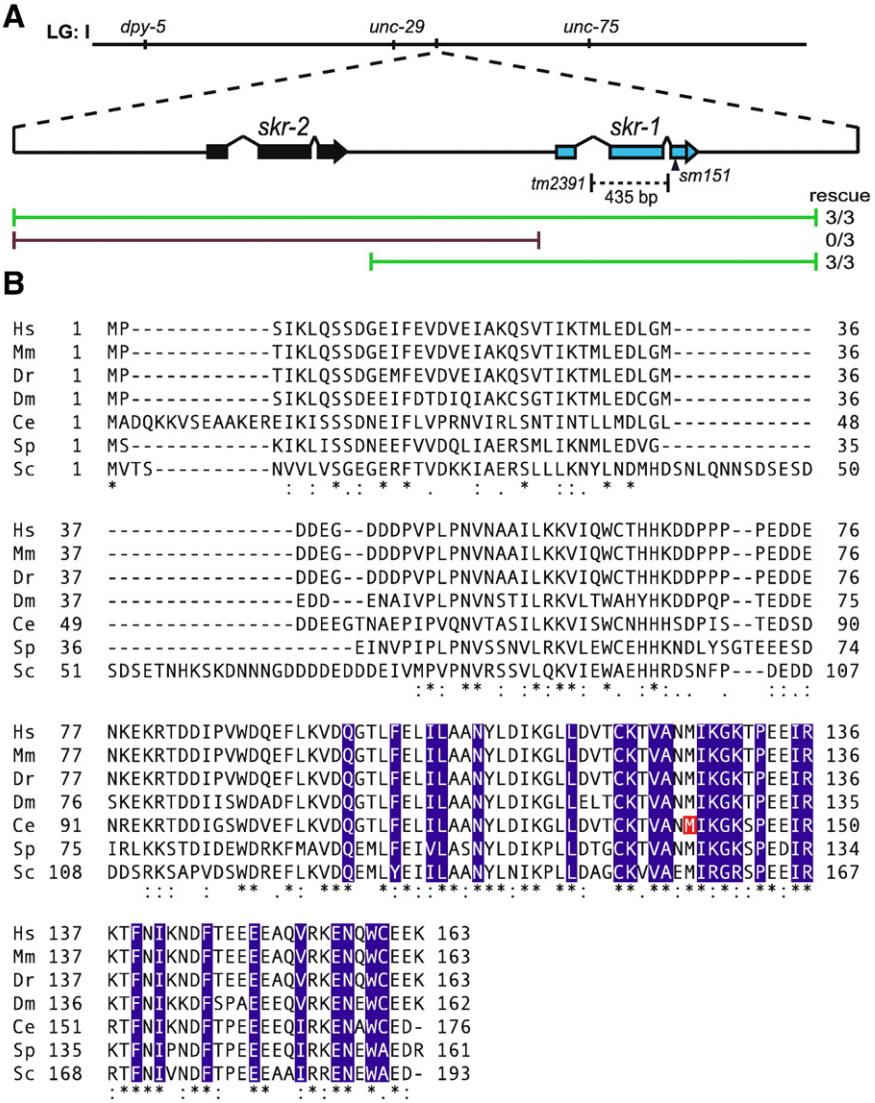

Fig. 2. (A) Schematic representation of the *skr-2* and *skr-1* locus of chromosome I (LG: I). The *skr-1(sm151)* lesion is indicated by an arrowhead and the *skr-1(tm2391)* deletion is indicated by a dashed line. Green lines represent PCR fragments that rescued *skr-1(sm151)* and the red lines represent PCR fragments that did not rescue. (B) Alignment was performed between Skp1-related proteins in human (Hs), mouse (Mm), zebrafish (Dr), fruitfly (Dm), worm (Ce), fission yeast (Sp), and budding yeast (Sc) with ClustalW2 (European Bioinformatics Institute of the European Molecular Biology Laboratory). (*) identical residues, (:) conserved residues, (.) semi-conserved residues. *C. elegans* SKR-1 M140 is indicated in red. F-box binding residues of human Skp1 are indicated in blue based on Schulmann et al. (2000).

(n1074) were introduced by PCR-mediated site-directed mutagenesis. Each cDNA entry clone was used to generate yeast two-hybrid vectors by Gateway reactions with pDEST22 (Gal4 Activation Domain, Trp selection) and pDEST32 (Gal4 DNA-binding domain, Leu selection; Invitrogen, Carlsbad, CA). Yeast Mav203 (MATα, *leu*2-3,112, *trp*1-190, *his*3Δ200, *ade*2-101, *gal*4Δ, *gal*80Δ, *SPAL*10::*URA*3, *GAL*1::*lacZ*, $HIS3_{UAS\ GAL1::}$*HIS*3@*LYS*2, *can*1^R, *cyh*2^R; Invitrogen, Carlsbad, CA) cells were co-transformed with the relevant plasmids to be tested for interaction. Yeast strains bearing test plasmids were replica plated onto -Leu-Trp-Ura plates, -Leu-Trp plates containing 3-Amino-1,2,4-Triazole (3AT; 10 mM, 25 mM, 50 mM, 75 mM, and 100 mM), -Leu-Trp plates with 0.2% 5-Fluoroacetic Acid (5FOA), and –Leu–Trp plates. Strains were plated in a dilution series (0.5×) starting with OD_{600} = 10. A positive interaction drives expression of the *lacZ*, *HIS3,* and *URA3* reporter genes that result in growth on -Ura and 3AT, and failure to grow in the presence of 5FOA. *lacZ* reporter expression was assayed by X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) staining of yeast transferred from -Leu-Trp plates to nitrocellulose filters and quantification of interactions was carried out by Chlorophenol red-β-D-galactopyranoside (CPRG; Invitrogen ProQuest Two-Hybrid System, Carlsbad, CA). In all assays, protein-protein interactions were tested with reciprocal Gal4 Activation domains (AD) and Gal4 DNA-binding domains (DB) and results were similar in both directions.

Western blotting

10 ml cultures of yeast strains expressing AD-SKR-1 or AD-SEL-10 were grown to an OD_{600} of 0.5 centrifuged. Yeast cell pellets were lysed with glass beads (200 μm diameter) by vortexing and boiling in SDS buffer with 0.2% β-mercaptoethanol. Samples were centrifuged to pellet cellular debris and supernatants were run on a 10% SDS-PAGE gel. The gel was transferred to a nitrocellulose membrane at 40 V overnight. Blots were blocked with 5% milk PBS + 0.1% Tween 20. Primary antibody was incubated for 2 h at room temperature followed by three 10-minute washes steps and secondary antibody was incubated for 1 h at room temperature followed by three 10-minute washes. Primary antibodies were anti-Gal4 AD (1:3000; gift of G. Odorizzi lab), and loading control was anti-phosphoglycerate kinase (PGK) monoclonal antibody (1:3000; Invitrogen, Carlsbad, CA). Secondary antibody was Goat anti-mouse HRP (1:3000; Bio-Rad, Hercules, CA). Visualization was performed with SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, Il) and X-ray film exposure.

Results

The sm151 mutation suppresses the sex-specific defects of sel-10(n1074)

We conducted a *sel-10(n1074)* suppressor screen (see Materials and methods) to understand how *sel-10* is involved in regulating the sex-determination pathway in *C. elegans*. From nearly 22,000 mutagenized haploid genomes, we isolated 5 extragenic *sel-10 (n1074)* suppressors, which suppress either the Egl phenotype, or the improperly surviving CEM phenotype in hermaphrodites, or both. One suppressor mutation, *sm151*, was further characterized because it is a relatively strong suppressor. Compared with *sel-10(n1074)* hermaphrodite animals in which 86% of CEMs improperly survive and 99% of HSNs inappropriately undergo apoptosis, *sm151*; *sel-10 (n1074)* double-mutant hermaphrodites have only 14% CEM survival and HSN survival is increased to 38% (Table 1). Masculinization of *sel-10(n1074)* hermaphrodites is also manifested in the male-specific coelomocyte positioning (64% animals) and the male-specific B cell morphology (92% animals) (Desai and Horvitz, 1989; Sulston and Horvitz, 1977). *sm151* partially suppresses both phenotypes, suggesting that its suppression of the *sel-10(n1074)* phenotypes is not restricted to sex-specific apoptosis (Table 1). Importantly, *sm151* does not suppress the sex-specific cell death defect caused by partial loss-of-function mutations in *tra*-1 or *tra-2*, or the inappropriate HSN death phenotype in *egl-1(n1084)* hermaphrodites (Table 2). Furthermore, *sm151* hermaphrodites and males alone do not exhibit any defects in sex-specific apoptosis or sex-determination (Table 1). Together these results suggest that *sm151* is a specific suppressor of *sel-10(n1074)*.

Table 1
Reduction of the *skr-1* activity suppresses *sel-10(n1074)* masculinization phenotypes

Genotype	% CEM	% HSN	% Male B cell morphology[a]	% Male coelomocyte position[b]
+	0	100	0	0
+ male	100	0	100	100
sel-10(n1074)	86	1	92	64
skr-1(sm151); sel-10(n1074)	14	38	50	11
skr-1(sm151)	0	100	0	0
skr-1(sm151) male[c]	100	0	ND	ND
skr-1(sm151)/+; sel-10(n1074)[d]	62	3	ND	ND
skr-1(tm2391)/+; sel-10(n1074)[e]	43	8	ND	ND
skr-1(sm151)/skr-1(tm2391); sel-10(n1074)[f]	1	77	ND	ND

All strains have XX karyotype, unless otherwise indicated (XO male), and carry *smIs26*, which harbors $P_{pkd\text{-}2}$*gfp*, $P_{tph\text{-}1}$*gfp*, and *unc-76* rescuing plasmid. *n* = 400 for CEMs, 100 for HSNs, and 50 for both B cell and coelomocyte scoring. Scoring of CEMs, HSNs, B cells and coelomocytes is described in Materials and methods. ND = not determined.
[a] Male B cell morphology: B cells in L1 larvae with enlarged nucleoli.
[b] Male coelomocyte position: one left ventral coelomocyte located posterior to the L1 somatic gonad primordium.
[c] Actual genotype *skr-1(sm151)*; *smIs26*; *him-5(e1490)*
[d] Actual genotype *skr-1(sm151)/dpy-5(e61) unc-29(e193)*; *smIs26*; *sel-10(n1074)*.
[e] Actual genotype *skr-1(tm2391)/dpy-5(e61) unc-29(e193)*; *smIs26*; *sel-10(n1074)*.
[f] Actual genotype *skr-1(tm2391)/dpy-5(e61) skr-1(sm151)*; *smIs26*; *sel-10(n1074)*.

sm151 causes a missense mutation in a C. elegans Skp1-related protein

Since *sm151* is a good *sel-10(n1074)* suppressor and does not cause any discernable phenotypes on its own, we cloned the gene affected by *sm151* to determine how it functions with *sel-10* (see Materials and methods). Briefly, we mapped *sm151* near the loci of two *C. elegans* Skp1-related genes, *skr-1* and *skr-2*, through three-factor mapping. Since Skp1 is known to function in a complex with F-box/WD40-repeat proteins such as CDC4, the mammalian homolog of SEL-10 (Cardozo and Pagano, 2004), and since SKR-1 interacts with SEL-10 based on the yeast two-hybrid assay (Yamanaka et al., 2002), we tested whether genomic fragments containing *skr-1* and *skr-2* can rescue *n1074* suppression by *sm151* (Fig. 2A). A long PCR product containing *skr-1,* but not *skr-2,* was able to reverse the suppression of the *sel-10(n1074)* phenotypes by *sm151*. A long PCR product containing *skr-2*, but not *skr-1*, failed to reverse the suppression. We sequenced the *skr-1* locus from *sm151*; *sel-10(n1074)* animals and found a single nucleotide change that results in a Methionine to Isoleucine substitution at amino acid 140. M140 is a completely conserved residue in Skp1-related proteins across diverse species (Fig. 2B). Skp1 binds to F-box proteins and M140 is adjacent to predicted F-box binding residues in mammalian Skp1 (residues shaded in blue in Fig. 2B; Schulman et al., 2000), suggesting that M140I substitution may compromise the ability of SKR-1 to bind F-box proteins.

skr-1(sm151) is a weak loss-of-function allele of skr-1

To determine the nature of the *skr-1(sm151)* mutation, we conducted a genetic analysis of *skr-1*. *skr-1(sm151)* does not have any discernable phenotypes on its own but RNAi-mediated knockdown of *skr-1* causes lethality (Nayak et al., 2002; Yamanaka et al., 2002), suggesting that *sm151* might be a weak allele. However, given the high sequence homology between *skr-1* and *skr-2,* it is possible that *skr-1(RNAi)* may cause a knockdown of both *skr* genes, which

Table 2
skr-1(sm151) does not suppress *tra-1(lf)*, *tra-2(lf)*, or *egl-1(gf)*

Genotype	% CEM	% HSN	% Tra	*n*
+	0	100	0	100
+ male	100	0	100	100
skr-1(sm151)	0	100	0	100
tra-1(e1488)	99	0	100	100
skr-1(sm151); tra-1(e1488)	99	0	100	100
tra-2(n1106)	76	7	17	100
skr-1(sm151); tra-2(n1106)	79	7	15	100
egl-1(n1084)	0	0	ND	50
skr-1(sm151); egl-1(n1084)	0	0	ND	50

All strains carry *smIs26* and have XX karyotype, unless otherwise indicated (XO male). The Tra phenotype refers to hermaphrodite animals displaying evident male tail morphology. ND = not determined.

leads to lethality. We thus screened for and obtained a deletion allele (*tm2391*) of *skr-1* (see Materials and methods). *tm2391* removes the entire second exon of *skr-1* as well as part of the first and second introns and is a predicted null allele. This deletion does not disrupt the neighboring *skr-2* gene (Fig. 2A). *skr-1(tm2391)* animals can not be maintained as a homozygous strain due to embryonic and larval lethality (data not shown). Rare *skr-1(tm2391)* homozygous escapers become uncoordinated sterile adults with hyperplasia of several tissues including the uterus and the spermatheca of the somatic gonad (Fig. 3). The lethality and hyperplasia phenotypes of *skr-1(tm2391)* animals are similar to those reported for animals treated with *skr-1/skr-2 RNAi* (Nayak et al., 2002). However, since *skr-1(tm2391)* disrupts *skr-1* but not *skr-2*, we conclude that *skr-1* and *skr-2* do not have redundant functions and that loss of *skr-1* is responsible for the observed lethality, hyperplasia, and sterility defects of *skr-1/skr-2 RNAi* treated animals. The *skr-1(tm2391)* hyperplasia phenotype is similar to that seen in animals deficient in *cul-1* or *lin-23,* which encodes a *C. elegans* Cullin and an F-box protein with WD40-repeats, respectively (Kipreos et al., 2000, 1996). Hyperplasia of post-embryonic tissues in *lin-23* and *cul-1* mutants is due to a requirement of SCF^{LIN-23} to degrade cell cycle regulators in response to developmental cues (Kipreos et al., 2000, 1996). It has been suggested that *lin-23* and *cul-1* function together with *skr-1* and/or *skr-2* to promote degradation of cell cycle regulators (Nayak et al., 2002). Therefore, our results suggest that SKR-1, but not SKR-2, likely plays the major role in mediating the functions of the SCF^{LIN-23} ubiquitin-ligase complex. Indeed, *skr-2* is expressed in intestine and *skr-1* is ubiquitously expressed (Yamanaka et al., 2002).

The stronger defects seen in the *skr-1(tm2391)* mutant suggests that *skr-1(sm151)* is a weak allele. However, since *sm151/+* weakly suppresses *sel-10(n1074)* masculinization phenotypes (Table 1), it remains possible that this semi-dominant suppression is the result of a gain-of-function or a neomorphic function of *skr-1*. We thus compared the extent of *sel-10(n1074)* suppression in *sm151/sm151*, *sm151/tm2391*, and *+/tm2391* backgrounds. *sel-10(n1074)* phenotypes are very sensitive to the dose of *skr-1*: *+/tm2391* suppresses to a greater extent than *+/sm151* and *sm151/tm2391* is a better suppressor than *sm151/sm151* (Table 1). Therefore, we conclude that *sm151* is a weak loss-of-function allele and that *sel-10(n1074)* masculinization is dependent on *skr-1(+)* activity. We were not able to assess the masculinization phenotypes of *skr-1(tm2391); sel-10(n1074)* animals due to the high penetrance of embryonic and larval lethality. Given that there are 21 *skr* genes in *C. elegans* (Nayak et al., 2002; Yamanaka et al., 2002), we cannot rule out the possibility that *sel-10(n1074)* masculinization phenotypes are also dependent on another *skr* gene.

Since *skr-1* encodes a member of the SCF complex (Nayak et al., 2002; Yamanaka et al., 2002), we surmised that loss of other SCF components might also suppress the *sel-10(n1074)* phenotypes. *cul-1* encodes a Cullin component of the SCF complex (Kipreos et al., 1996). CUL-1 also binds to SKR-1 (as well as other SKR proteins) in a yeast two-hybrid assay (Nayak et al., 2002; Yamanaka et al., 2002). Therefore we tested whether loss of *cul-1* could suppress *sel-10 (n1074)* masculinization. Due to the general sickness of *cul-1(e1756)* animals, we found that the presence or absence of the HSNs could not be reliably scored. However, the CEMs could be easily detected with a $P_{pkd\text{-}2}GFP$ reporter. The percentage of improperly surviving CEMs was significantly less in *cul-1(e1756); sel-10(n1074)* hermaphrodites than in *sel-10(n1074)* hermaphrodite animals (Table 3). This suggests that *sel-10(n1074)* masculinization is dependent upon an SCF complex that is composed of SKR-1, CUL-1 and SEL-10. The observation that the *cul-1(e1756)* strong loss-of-function allele did not completely suppress *sel-10(n1074)* masculinization likely reflects the fact that *cul-1(e1756)* homozygotes from heterozygous mothers exhibit partial *cul-1(+)* maternal rescue (Kipreos et al., 1996).

skr-1(sm151) genetically interacts with sel-10 in lin-12 activity assays

sel-10 was first identified as a regulator of LIN-12/Notch signaling (Hubbard et al., 1997), which is required for the anchor cell/ventral uterine precursor (AC/VU) cell fate decision (Greenwald et al., 1983; Kimble and Hirsh, 1979). Loss of *lin-12* causes two initially equivalent cells, Z1.ppp and Z4.aaa, to adopt an AC fate and is thus called a 2AC

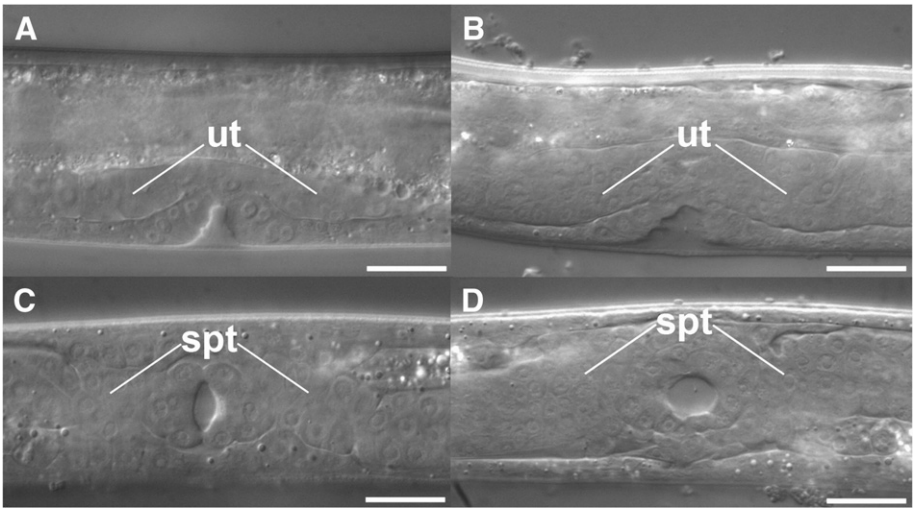

Fig. 3. Differential Contrast Interference (DIC) lateral views of (A) wild-type (N2) normal uterine development and (B) *skr-1(tm2391)* uterine hyperplasia (ut = uterus). DIC ventral views of (C) N2 normal somatic gonad development and **(D)** *skr-1(tm2391)* somatic gonad hyperplasia (sg = somatic gonad). Bars=25 μm.

Table 3
Loss of *cul-1* activity suppresses *sel-10(n1074)* masculinization phenotypes

Genotype	% CEM	*n*
+	0	100
+ male	100	100
cul-1(e1756)	0	100
sel-10(n1074)	76	100
cul-1(e1756); sel-10(n1074)	35	44
cul-1(e1756) male	100	20

All strains have XX karyotype, unless otherwise indicated (XO male), and carry *smIs23*, which harbors $P_{pkd\text{-}2}$*gfp*. *cul-1(e1756)* animals were the non-GFP progeny from *cul-1 (e1756)/hT2[GFP]* parents.

phenotype (Greenwald et al., 1983). LIN-12/Notch is also required for the vulval precursor cells (VPCs) to adopt proper fates (1° or 2° vulval fates, or a 3° non-vulval fate). Hyperactive *lin-12* activity results in all VPCs adopting 2° fates and thus over-induction of the vulval tissue, which is known as a Multivulva (Muv) phenotype (Sundaram and Greenwald, 1993).

Since SEL-10 is suggested to target LIN-12 for ubiquitin-mediated degradation, loss of *sel-10* would result in an increase in the level of LIN-12. Phenotypically, loss of *sel-10* suppresses the 2AC phenotype associated with *lin-12* reduction-of-function *(rf)* alleles and enhances the Muv phenotype of *lin-12* gain-of-function *(gf)* alleles (Hubbard et al., 1997). Although *sel-10(n1074)* has a dominant effect on sex-determination, *sel-10(n1074)* is a recessive reduction-of-function mutation with respect to *lin-12* activity assays (Jager et al., 2004). Unlike *sel-10(n1074)*, which suppresses the *lin-12(rf)* 2AC phenotype from 80% to 25%, *skr-1(sm151)* did not suppress the 2AC phenotypes of the *lin-12(rf)* animals on its own, but further reduced the 2AC phenotype of *lin-12(rf); sel-10(n1074)* animals from 25% 2AC to 18% 2AC (Table 4). Importantly, *skr-1(sm151)* did not confer additional suppression of the *lin-12(rf)* 2AC phenotype in a *sel-10(ok1632)* null background (see Materials and methods), which has 19% 2AC. The effect of *skr-1(sm151)* in the *lin-12(rf)* assay is small and can only be detected in the *n1074* background. Similarly, we did not observe an enhancement of the *lin-12(gf)* Muv phenotype by *skr-1(sm151)*. However, we found that *skr-1(sm151)* greatly enhances the Muv phenotype of *lin-12(gf); sel-10(n1074)* animals from 31% to 88% and does not enhance the Muv phenotype of *lin-12(gf); sel-10(ok1632)* (Table 5).

Since *skr-1(sm151)* had no effect on the *lin-12(rf)* or *lin-12(gf)* phenotype in *sel-10(+)* genetic backgrounds, *skr-1(sm151)* may be a very weak allele and the phenotypic assays are not sensitive enough to detect a difference. Alternatively, *skr-1(sm151)* could be allele-specific to *sel-10(n1074)*. To distinguish between these two possibilities, we

Table 4
skr-1(sm151) suppresses *lin-12(rf)* in a *sel-10(n1074)* background

Genotype	% 2 AC phenotype (25 °C)	*n*
skr-1(sm151)	0	100
skr-1(sm151); sel-10(n1074)	0	100
skr-1(sm151); sel-10(ok1632)	0	100
lin-12(ar170)	80	100
skr-1(sm151); lin-12(ar170)	77	100
lin-12(ar170); sel-10(n1074)	25*	200
lin-12(ar170); sel-10(ok1632)	19	100
skr-1(sm151); lin-12(ar170); sel-10(n1074)	18*	200
skr-1(sm151); lin-12(ar170); sel-10(ok1632)	18	100
skr-1(sm151); unc-76	0	100
skr-1(sm151); sel-10(ar41) unc-76	0	100
lin-12(ar170); unc-76	79	100
skr-1(sm151); lin-12(ar170); unc-76	81	100
lin-12(ar170); sel-10(ar41) unc-76	21	100
skr-1(sm151); lin-12(ar170); sel-10(ar41) unc-76	19	100

ACs were scored with DIC (100x) at the late L3/early L4 stage.
unc-76(e911) is linked to *sel-10(ar41)* and was included in relevant control strains.
* These values are statistically different based on Chi square analysis, $P<0.03$.

Table 5
skr-1(sm151) enhances *lin-12(gf)* in a *sel-10(n1074)* background

Genotype	% Muv (20 °C)	*n*
skr-1(sm151)	0	100
skr-1(sm151); sel-10(n1074)	0	100
skr-1(sm151); sel-10(ok1632)	0	100
lin-12(n379)	10	100
skr-1(sm151); lin-12(n379)	12	100
lin-12(n379); sel-10(n1074)	31	100
lin-12(n379); sel-10(ok1632)	96	100
skr-1(sm151); lin-12(n379); sel-10(n1074)	88	100
skr-1(sm151); lin-12(n379); sel-10(ok1632)	97	100
skr-1(sm151); unc-76	0	100
skr-1(sm151); sel-10(ar41) unc-76	0	100
lin-12(n379); unc-76	11	100
skr-1(sm151); lin-12(n379); unc-76	12	100
lin-12(n379); sel-10(ar41) unc-76	59	100
skr-1(sm151); lin-12(n379); sel-10(ar41) unc-76	57	100

The Muv phenotype was scored in adult animals using DIC (40×).
unc-76(e911) is linked to *sel-10(ar41)* and was included in relevant control strains.

tested the effect of *skr-1(sm151)* on the *lin-12(rf)* or *lin-12(gf)* phenotype in another *sel-10* mutant background, *sel-10(ar41)*. *sel-10 (ar41)* contains a stop codon before the WD40-repeats but does not behave genetically as a null mutation (Hubbard et al., 1997; Table 5), possibly due to read through of the stop codon. In the *lin-12(gf)* phenotypic assay, *sel-10(ar41)* does not enhance the Muv phenotype as well as *sel-10(ok1632null)* (Table 5), again indicating that *sel-10 (ar41)* probably is not a null allele.

In these *lin-12* activity assays, *skr-1(sm151)* did not have any significant effect on the *lin-12(rf)* or *lin-12(gf)* phenotypes in a *sel-10 (ar41)* background. Although there is little room for further suppression of the *lin-12(rf)* 2AC phenotype by *skr-1(sm151)* in *lin-12(rf); sel-10(ar41)* animals compared with *lin-12(rf); sel-10(ok1632null)* animals (21% to 19%; Table 4), there is a significant difference in the percentage of the Muv phenotype between *lin-12(gf); sel-10(ar41)* (59%) and *lin-12(gf); sel-10(ok1632)* (96%) animals, indicating that the Muv phenotype of *lin-12(gf); sel-10(ar41)* can be enhanced (Table 5). The fact that *skr-1(sm151)* can enhance the effect of *sel-10(n1074)* but not that of *sel-10(ar41)* in the *lin-12(rf)* or *lin-12(gf)* assay is consistent with *skr-1(sm151)* being an allele-specific suppressor/enhancer of *sel-10(n1074)*. However, additional non-null alleles of *sel-10* are not available to further test this hypothesis.

skr-1(sm151) does not genetically interact with lin-23

There are many F-box proteins in *C. elegans*, including SEL-10 and LIN-23, both of which also contain WD40 repeats. The observation that *skr-1(tm2391)* phenocopies *lin-23(null)* mutants (Fig. 3) prompted us to test whether *skr-1(sm151)* could enhance the defect caused by *lin-23* mutations. Since there are no partial loss-of-function *lin-23* alleles that cause hyperplasia, we tested instead whether *skr-1 (sm151)* could enhance the defect of *lin-23(ot1)* animals, which are deficient in AVL GABAergic neuron axon outgrowth as assayed by the *unc-47::GFP* reporter (Mehta et al., 2004). No enhancement of *lin-23 (ot1)* AVL axonal outgrowth defect was seen, suggesting that *skr-1 (sm151)* activity is sufficient for *lin-23* (Table 6). However, *lin-23(ot1)* is almost null for the AVL axon defects and leaves little room for enhancement. Therefore we tested whether *skr-1(sm151)* could cause an AVL axon defect in a *lin-23(ot1)*/+ genetic background. We did not see any effect of *skr-1(sm151)* in this background either (Table 6). Taken together, *skr-1(sm151)* does not have a detectable effect on the processes regulated by *lin-23*.

SKR-1(M140I) and SEL-10(G567E) reduce SKR-1/SEL-10 binding

Since SKR-1(M140I) alters a residue that is within the F-box interaction region of SKR-1, SKR-1(M140I) may affect SKR-1 binding to

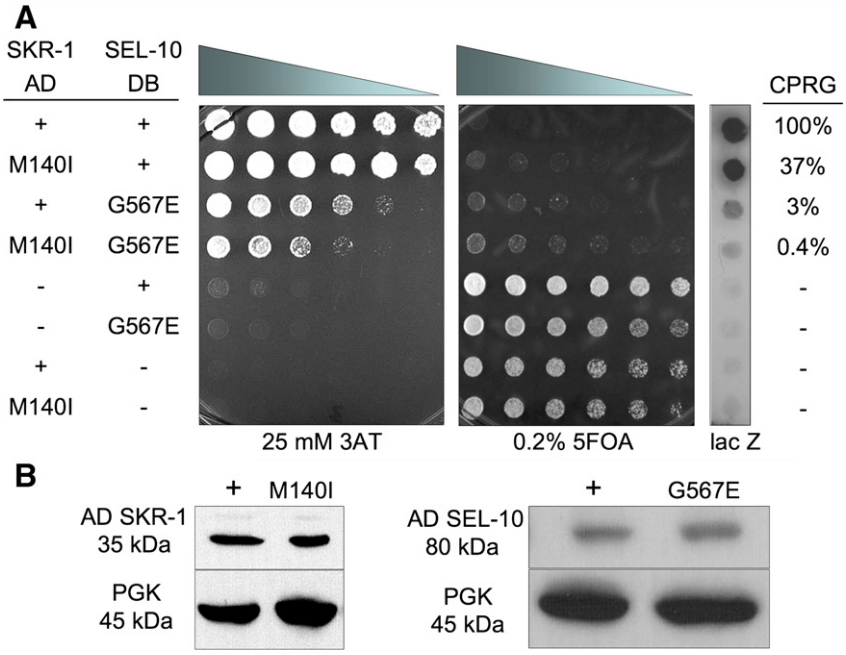

Fig. 4. (A) A yeast two-hybrid assay with AD-SKR-1, DB-SEL-10, and the respective M140I and G567E mutants or empty vectors (−). Yeast were plated in a 0.5× dilution series from left to right starting at OD600 = 10. Positive interactions exhibit increased growth on 3AT and decreased growth on 5FOA relative to negative controls (rows 5–8). Positive interactions are shown by lacZ staining. β-galactosidase activity based on the CPRG assay is shown as a percentage of the activity observed in yeast expressing SKR-1(+)/SEL-10(+). Yeast were grown for 2 days before imaging. (B) Western blot analysis of yeast lysates containing AD-SKR-1 and AD-SKR-1(M140I) (left) and AD-SEL-10 and AD-SEL-10(G567E) (right). Phosphoglycerate kinase (PGK) was used as a loading control. See Materials and methods for additional details.

SEL-10. We used the yeast two-hybrid assay to assess the binding of the SKR-1 proteins (wild-type and M140I) to SEL-10 as described previously by Yamanaka et al. (2002). We found a strong protein interaction between GAL4 activation domain (AD) SKR-1 fusion (AD-SKR-1) and GAL4 DNA-binding domain (DB) SEL-10 fusion (DB-SEL-10) based on growth on 3-Amino-1,2,4-Triazole (3AT), inhibition of growth on 0.2% 5-fluoroorotic acid (5FOA), and lacZ staining (see Materials and methods; Fig. 4A). DB-SKR-1 and AD-SEL-10 also interact strongly as revealed by the same assays but the interaction is slightly weaker in all assays, particularly in growth inhibition on 0.2% 5FOA and lacZ staining (data not shown). A quantitative measurement of the β-galactosidase activity was performed with a chlorophenolred-β-D-galactopyranoside (CPRG) assay (see Materials and methods) and we found that the SKR-1(M140I)/SEL-10 interaction is 37% as strong as SKR-1/SEL-10 in this assay (Fig. 4A). This suggests that SKR-1(M140I) reduces SKR-1 binding to SEL-10.

We also tested the interaction between SKR-1(WT or M140I) and SEL-10(G567E) proteins and found that SKR-1(M140I) had significantly reduced binding to SEL-10(G567E) compared to the SKR-1/SEL-10(G567E) interaction in all three yeast two-hybrid assays (Fig. 4A). Surprisingly, SEL-10(G567E) binds only weakly to SKR-1 compared to the SEL-10/SKR-1 interaction (3% of the SEL-10/SKR-1 interaction; Fig. 4A). The F-box motif mediates the interaction between F-box proteins and the Skp1 proteins (Bai et al., 1996). Intriguingly, the F-box of SEL-10 is located at the amino terminal region, whereas the G567E mutation locates at the SEL-10 carboxyl terminal tail. Based on the structures of SCF complexes containing the mammalian SEL-10 homologues (Hao et al., 2007; Orlicky et al., 2003), the C-terminal tails of F-box proteins do not contact Skp1. We thus examined if the G567E mutation may affect the stability of SEL-10 in yeast by performing western blot analysis (see Materials and methods) on yeast lysates to determine the expression levels of SEL-10, SEL-10 (G567E), SKR-1, and SKR-1(M140I). We did not detect any significant difference in protein expression of the wild-type versus the mutant forms of SEL-10 and SKR-1 (Fig. 4B). Therefore we conclude that the M140I mutation in SKR-1 and the G567E mutation in SEL-10 both reduce SKR-1/SEL-10 binding. Our data suggest that the C-terminus of SEL-10 is important for binding to SKR-1, which is an unexpected finding that has not been reported previously. Despite greatly reduced binding between SKR-1(M140I) and SEL-10(G567E) (0.4% of the SKR-1/SEL-10 interaction), SKR-1(M140I)/SEL-10(G567E) still interact at a level well above background in the yeast two-hybrid system and may retain some activity in promoting degradation of their target protein(s) *in vivo* (Fig. 4A).

We also tested if the M140I mutation disrupts SKR-1 binding to another SCF component, CUL-1 (Yamanaka et al., 2002; Nayak et al., 2002). We found no significant difference between the binding of SKR-1/CUL-1 and SKR-1(M140I)/CUL-1 in all yeast two-hybrid assays (Fig. 5 and data not shown). We conclude that the SKR-1 M140I mutation specifically interferes with the binding to SEL-10, especially the binding to the SEL-10(G567E) protein, which underlies the strong suppression of the *sel-10(n1074)* defects by *skr-1(sm151)*.

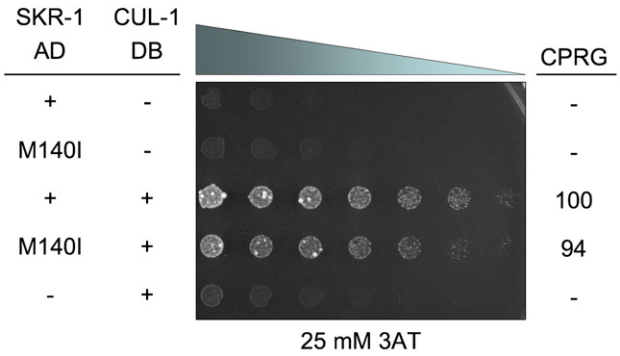

Fig. 5. A yeast two-hybrid assay with AD-SKR-1 or AD-SKR-1(M140) and DB-CUL-1 or empty vectors (−). Yeast were plated in a 0.5× dilution series from left to right starting at OD600 = 10. Positive interactions exhibit increased growth on 3AT relative to negative controls (rows 1, 2, and 5). β-galactosidase activity based on CPRG assay is shown as a percentage of the activity observed in yeast expressing SKR-1(+)/CUL-1(+). Yeast were grown for 2 days before imaging. See Materials and methods for additional details.

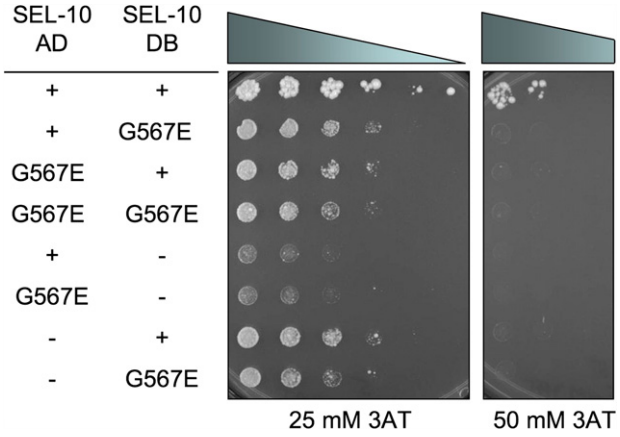

Fig. 6. A yeast two-hybrid assay with AD-SEL-10 and DB-SEL-10. Yeast were plated in a 0.5× dilution series from left to right starting at OD600 = 10. Positive interactions exhibit increased growth on 3AT relative to negative controls (rows 5–8). Yeast were grown for 7 days before imaging. See Materials and methods for additional details.

The SEL-10 G567E mutation disrupts the formation of SEL-10 homodimers

The yeast SCF^{CDC4} and mammalian SCF^{Fbw7} complexes form dimers that are important for the function of the complex as an E3 ubiquitin ligase (Hao et al., 2007; Tang et al., 2007). The WD40-repeat F-box proteins CDC4, human β-TrCP, and *C. elegans* SEL-10 all contain a conserved D-domain, just N-terminal to the F-box domain, that is required for dimerization (Tang et al., 2007). We thus tested if SEL-10 interacts with itself by the yeast two-hybrid analysis. We found that SEL-10 does self interact, possibly by forming homodimers, and that the G567E mutation disrupts SEL-10 dimerization (Fig. 6). Therefore, *sel-10(n1074)* may cause reduction of *sel-10* activity and the activity of the SCF^{SEL-10} complex by disrupting SEL-10 dimerization.

Discussion

SKR-1 is a critical component of the C. elegans SCF^{SEL-10} and SCF^{LIN-23} complexes

The SCF complex is a multi-subunit E3 ubiquitin ligase, conserved from yeast to humans, that attaches polyubiquitin chains to its protein substrates and targets them for degradation by the proteasome. In yeast and humans, Skp1, Rbx, and Cul1 are the common components and the variable F-box proteins provide substrate specificity (Kipreos and Pagano, 2000). Interestingly, in yeast and humans, there is a single Skp1 gene but in *C. elegans* there are 21 Skp1-related (*skr*) genes (Nayak et al., 2002; Yamanaka et al., 2002). The reason for so many *skr* genes in *C. elegans* is not clear.

In this study, we present genetic evidence suggesting that SKR-1 is the Skp1 protein for two major *C. elegans* SCF complexes, SCF^{SEL-10} and SCF^{LIN-23}, and that it is not redundant with other SKR proteins. The F-box protein SEL-10 is involved in the ubiquitin-mediated degradation of LIN-12 in the AC/VU decision and vulval induction (Hubbard et al., 1997) and has been proposed to promote degradation of FEM-1 and FEM-3 in sex determination (Jager et al., 2004). We show that SKR-1 is a critical component of the SCF^{SEL-10} complex that regulates the activity of these pathways. First, SEL-10 and SKR-1 physically interact in yeast 2-hyrid assays, whereas other SKR proteins do not interact with SEL-10 (Yamanaka et al., 2002). Second, our genetic analysis suggests that the role of *sel*-10 in sex determination is dependent on *skr-1(+)* activity: *skr-1(sm151)* or *skr-1(sm151)/skr-1(tm2391)* suppresses masculinization of hermaphrodites caused by *sel-10(n1074)* (Table 1). In addition, *skr-1(sm151)* can further suppress the 2AC defect of the *lin-12(rf)*; *sel-10(n1074)* mutant and enhance the Muv defect of the *lin-12(gf)*; *sel-10(n1074)* mutant (Table 4). It remains possible that the SCF^{SEL-10} complex uses SKR-1 in some cells and other SKR proteins in other cell types. However, we find this unlikely since SKR-1::GFP is widely expressed in *C. elegans* (Yamanaka et al., 2002) and our genetic analysis of *skr-1* and *sel-10* interactions includes several different cell types such as HSN and CEM neurons, the AC/VU cells, and P.np cells, which become either vulval tissue or hypodermis. Since a loss-of-function mutation in *cul-1* suppresses masculinization of hermaphrodites caused by *sel-10(n1074)* (Table 3), this result suggests that CUL-1 is also a component of the SCF^{SEL-10} complex.

In addition to SEL-10, LIN-23 is another well-described F-box protein in *C. elegans*. *lin-23* promotes cell cycle exit during *C. elegans* development and appears to work in conjunction with *cul-1* based on their similar loss-of-function phenotypes such as hyperplasia of several tissues (Kipreos et al., 2000). It has been suggested that both *skr-1* and *skr-2* may work with *lin-23* and *cul-1* to regulate cell cycle exit (Nayak et al., 2002), since both SKR-1 and SKR-2 bind CUL-1 in a yeast 2-hybrid assay. However, the strong sequence similarity between *skr-1* and *skr-2* prevents dissection of their respective contributions by RNAi, which is predicted to knockdown both genes (Nayak et al., 2002). Our observation that a null allele of *skr-1* results in hyperplasia of several tissues similar to that seen in *lin-23* and *cul-1* loss-of-function mutants (Fig. 3) establishes that SKR-2 could not substitute for SKR-1 in the SCF^{LIN-23} complex and that SKR-1 likely is the major, if not the only, SKR component for the SCF^{LIN-23} complex.

skr-1(sm151) is a specific suppressor/enhancer of sel-10(n1074)

Although *skr-1(sm151)* was isolated as a strong suppressor of the masculinization phenotype caused by the semi-dominant *sel-10 (n1074)* mutation, *skr-1(sm151)* does not suppress the masculinization phenotype caused by loss-of-function mutations in *tra-1* or *tra-2*. The *skr-1(sm151)* mutant by itself is superficially wild-type and does not display any detectable defects. Interestingly, *skr-1(sm151)* specifically enhances the Muv defect of the *lin-12(gf)*; *sel-10(n1074)* mutant but not that of the *lin-12(gf)*; *sel-10(ar41)* mutant, which contains a *sel-10* strong loss-of-function allele, but not a null allele (Table 5). In addition, *skr-1(sm151)* does not enhance or suppress the AVL neuron axonal outgrowth defect caused by a loss-of-function mutation in another closely related F-box gene, *lin-23*. These results together suggest that *skr-1(sm151)* is likely a mutation that specifically suppresses/enhances the defect of *sel-10(n1074)* animals in sex-determination and *lin-12* activity assays. There are no other non-null *sel-10* alleles available for testing whether *skr-1(sm151)* is truly an allele-specific suppressor/enhancer of *sel-10(n1074)*.

Paradoxically, complete loss of the *sel-10* activity also results in weak masculinization of hermaphrodites (Jager et al., 2004), suggesting that the stronger masculinization phenotype caused by *sel-10 (n1074)* is unlikely a result of increased SEL-10 activity but rather a gain of new function for the SEL-10(G567E) protein. One hypothesis is that there are redundant F-box proteins that target FEM-1 and FEM-3

Table 6
skr-1(sm151) does not show genetic interactions with *lin-23*

Genotype	% AVL defects	*n*
+	0	100
skr-1(sm151)	1	100
lin-23(ot1)	93	100
skr-1(sm151); lin-23(ot1)	99	100
lin-23(ot1)/+	0	50
lin-23(ot1)/+; skr-1(sm151)/+	0	50
lin-23(ot1)/+; skr-1(sm151)	0	50

lin-23(ot1) animals show defects in AVL axon outgrowth as assayed by *unc-47::GFP*. All strains were scored as L4s with *oxIs12[unc-47::GFP]*.

Table 7
skr-1(sm151) and *cul-1(e1756)* do not enhance the masculinization defect caused by *sel-10(ok1632null)*

Genotype	% CEM	% HSN	*n*
sel-10(ok1632)	1	97	100
skr-1(sm151)	0	100	100
skr-1(sm151); sel-10(ok1632)	<1	97	100
cul-1(e1756)	0	ND	31
cul-1(e1756); sel-10(ok1632)	0	ND	26

All strains carry *smIs26*, except for strains bearing *cul-1(e1756),* which carry *smIs23*. ND = not determined.

for degradation (Jager et al., 2004), although there are no other known F-box proteins except SEL-10 that affect sex-determination. Loss of *sel-10* is thus compensated for by other F-box genes, which would explain why loss of *sel-10* results in a weak masculinization phenotype. On the other hand, the *sel-10(n1074)* mutation may result in the formation of a stable but non-functional $SCF^{SEL\text{-}10(G567E)}$ complex such that other redundant F-box proteins do not gain access to FEM-1 or FEM-3 (Jager et al., 2004). As a result, *sel-10(n1074)* causes a stronger masculinization defect. Since we isolated *skr-1(sm151)* as a suppressor of the *sel-10(n1074)* masculinization defect and the *sm151* (M140I) mutation greatly reduces SKR-1 binding to SEL-10(G567E), it is possible that the SKR-1(M140I)-containing $SCF^{SEL\text{-}10(G567E)}$ complex may now allow other F-box proteins to gain access to the complex and degrade FEM-1 and FEM-3. However, given that SEL-10(G567E) binds wild-type SKR-1 very poorly (Fig. 4A) and fails to form SEL-10 homodimers, it seems unlikely that SEL-10(G567E) would result in the formation of a stable, dominant-negative $SCF^{SEL\text{-}10(G567E)}$ complex. In addition, we find that neither *skr-1(sm151)* nor *cul-1(e1756)* can enhance the mild masculinization defect caused by *sel-10(ok1632null)* (Table 7), suggesting that there are no other F-box proteins forming E3 ligase complexes with SKR-1 and CUL-1 and acting redundantly with SEL-10 to regulate sex-determination. In fact, our results are more consistent with *sel-10(n1074)* causing the formation of an unstable, compromised $SCF^{SEL\text{-}10(G567E)}$ complex with reduced E3 ligase activity. Indeed, *sel-10(n1074)* behaves as a loss-of-function mutation with respect to the *lin-12* signaling (Tables 4 and 5) (Jager et al., 2004). Similarly, the $SCF^{SEL\text{-}10(G567E)}$ complex may have reduced E3 ligase activity towards all of its substrates, including FEM-1 and FEM-3.

The SEL-10 G567E mutation is located at the C-terminus inside the eighth WD40-repeat region, which is a site involved in substrate binding in homologous SCF complexes (Hao et al., 2007; Jager et al., 2004; Orlicky et al., 2003). It is possible that the G567E substitution alters SEL-10 substrate binding in a way that $SCF^{SEL\text{-}10(G567E)}$ targets a protein for degradation that normally is not recognized by $SCF^{SEL\text{-}10}$. In this case, such a neomorphic activity of the $SCF^{SEL\text{-}10(G567E)}$ complex may target a hermaphrodite-promoting, sex-determination factor for degradation. If this factor normally is not targeted for degradation, then even a small decrease in steady-state level of this protein may have a large impact on sex-determination. We note that even if the $SCF^{SEL\text{-}10(G567E)}$ complex is less stable due to reduced SKR-1/SEL-10 binding caused by the G567E mutation (Fig. 4A), any such degradation of its neomorphic target could have a significant effect in sex determination and could be suppressed by the SKR-1(M140I) mutation that further destabilizes the SCF complex.

The C-terminus of SEL-10

The SEL-10 G567E mutation is located at the C-terminus inside the eighth WD40-repeat region, a site that is predicted to be involved in substrate binding based on the structure of the Skp1/CDC4 complex and the structure of the Skp1/Fbw7 complex (Hao et al., 2007; Jager et al., 2004; Orlicky et al., 2003). The C-terminal tails of the F-box proteins appear to be away from Skp1 in these structures. It is thus surprising that the G567E mutation greatly reduces SEL-10 binding to SKR-1. Since G567E also disrupts SEL-10 dimerization, which has been shown to be important for the SCF activity (Tang et al., 2007), it is possible that SEL-10 dimerization is important for the SEL-10/SKR-1 interaction and that G567E impairs the SEL-10/SKR-1 binding by disrupting SEL-10 dimerization. The F-box of SEL-10 is presumed to confer SKR-1 binding and we confirmed that an F-box deletion prevents SKR-1/SEL-10 interaction (data not shown). The C-terminus of SEL-10 is thus required for efficient SKR-1 binding but is not sufficient for mediating SKR-1 binding in the absence of the F-box. Our study thus reveals a previously unreported role of the C-terminus of SEL-10 in stabilizing the interaction between a F-box protein and a Skp1 protein. It will be interesting to see if the C-terminus of other F-box proteins influences Skp1 binding.

Acknowledgments

We gratefully acknowledge Ning Zheng and Xuedong Liu for their advice and discussion, the technical assistance of Eric Griffiths, the *Caenorhabditis* Genetics Stock Center for strains, and Xue lab members for their discussions and comments on the manuscript. This work is supported by a NIH NRSA postdoctoral fellowship (5 F32 GM075612) to D.J.K., a Burroughs Wellcome Fund Career Award and NIH R01 grants (GM66262 and GM59083) to D.X., and a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan to S.M.

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